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The SSB-positive/SSA-negative antibody profile is not associated with key phenotypic features of Sjögren's syndrome

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Abstract

Objective—To determine whether the Sjögren's syndrome B (SSB)-positive/Sjögren's syndrome A (SSA)-negative antibody profile is associated with key phenotypic features of SS.

Methods—Among registrants in the Sjögren's International Collaborative Clinical Alliance (SICCA) with possible or established SS, we compared anti-SSA/anti-SSB reactivity profiles against concurrent phenotypic features. We fitted logistic regression models to explore the association between anti-SSA/anti-SSB reactivity profile and each key SS phenotypic feature, controlling for potential confounders.

Results—Among 3297 participants, 2061 (63%) had negative anti-SSA/anti-SSB, 1 162 (35%) had anti-SSA with or without anti-SSB, and 74 (2%) anti-SSB alone. Key SS phenotypic features were more prevalent and had measures indicative of greater disease activity in those participants with anti-SSA, either alone or with anti-SSB, than in those with anti-SSB alone or negative SSA/SSB serology. These between-group differences were highly significant and not explained by confounding by age, race/ethnicity or gender. Participants with anti-SSB alone were comparable to those with negative SSA/SSB serology in their association with these key phenotypic features. Among SICCA participants classified with SS on the basis of the American-European Consensus Group or American College of Rheumatology criteria, only 2% required the anti-SSB-alone test result to meet these criteria.

Conclusions—The presence of anti-SSB, without anti-SSA antibodies, had no significant association with SS phenotypic features, relative to seronegative participants. The solitary presence of anti-SSB antibodies does not provide any more support than negative serology for the diagnosis of SS. This serological profile should thus be interpreted cautiously in clinical practice and potentially eliminated from future classification criteria.

Anti-Sjögren's syndrome A (SSA) (Ro) and anti-Sjögren's syndrome B (SSB) (La) antibodies are present in up to 75% of patients with primary SS.[12] Two profiles of anti-SSA/anti-SSB reactivity are common, both anti-SSA and anti-SSB and anti-SSA alone, with the former being more common than the latter.[34] Anti-SSB alone is an uncommon serological profile in established SS[35–8] but often prompts an SS evaluation in patients with sicca symptoms. It may reflect the reported 1–15% prevalence of anti-SSB alone in healthy individuals[7910] and the increased sensitivity of current solid-phase immunoassays.[11]

Anti-SSA/anti-SSB serology is a criterion for the classification of SS in both the American-European Consensus Group (AECG) and American College of Rheumatology (ACR) sets.[1213] However, it is not known whether the solitary presence of anti-SSB has validity equivalent to anti-SSA, present either alone or with anti-SSB, in supporting SS classification. Accordingly, we sought to determine the association of three different anti-SSA/anti-SSB serological profiles with SS phenotypic features among participants in the Sjögren's International Collaborative Clinical Alliance (SICCA) registry.[14]

METHODS

SICCA cohort and study population

SICCA is a registry of individuals with symptoms or signs indicative of possible, early to well-established SS, each of whom underwent a systematic and extensive evaluation for SS using uniform protocol-driven data collection methods.[14] Methodological details of this registry are provided in the online supplementary materials. There were 3514 SICCA participants enrolled as of 6 September 2013. We excluded 217 for whom data were lacking on at least one of the three objective criteria for SS, as defined by the ACR criteria,[4] leaving 3297 participants for the current cross-sectional analyses.

All SICCA serological testing was performed centrally by Quest Diagnostics (Madison, New Jersey, USA). For the initial 876 registrants, anti-SSA and anti-SSB were tested using the Bio-Rad Autoimmune EIA (Bio-Rad, Hercules, California, USA), a semiquantitative enzyme immunoassay (EIA) that used purified native antigens and reported results in either enzyme units or index values. The subsequent 2421 registrants had anti-SSA/anti-SSB testing performed with the newly introduced Bio-Rad Bioplex 2200 multiplex flow immunoassay (MFIA). For this assay, the Ro52 antigen was recombinant, while Ro60 and SSB were native in origin.[15] Positive results were expressed in ‘antibody index’ (AI) units and provided as continuous variable measures up to 8 AI.

Statistical analysis

Proportions for categorical variables and median (range) for continuous variables were used to describe the sociodemographic features in the cohort. We used Fisher’s exact tests to evaluate associations between categories of anti-SSA/anti-SSB serological reactivity (anti-SSA with or without anti-SSB, only anti-SSB, and lacking both anti-SSA and anti-SSB at baseline) and key SS phenotypic features. Rank-sum tests were used to compare the continuous SS phenotypic measures between the three groups (Kruskal-Wallis) and for pairwise comparisons between selected groups of interest (Wilcoxon) defined by serological reactivity.

We used logistic regression models to further investigate the association between anti-SSA/anti-SSB serological profile (included as the primary predictor) on each key SS phenotypic feature (treated as binary outcomes), controlling for potential confounders, and allowing for the possibility of interaction between the presence of anti-SSA and of anti-SSB. Confounding variables included age (in years), sex and race (with the Caucasian subgroup as reference). All statistical analyses were performed using SAS (V9.3, SAS Institute, Cary, North Carolina, USA) and Stata (VI3.1, StataCorp, College Station, Texas, USA).

RESULTS

The sociodemographic characteristics of the 3297 participants are shown in table 1. A total of 1490 (45%) met the ACR criteria (129 with secondary SS) and 1457 (44%) met the AECG criteria for SS (119 with secondary SS).

The majority of participants had negative SSA/SSB serology, 35% had anti-SSA with or without anti-SSB and 2% had only anti-SSB (table 2). The anti-SSB alone serological profile was more prevalent among the participants who had their testing performed with MFIA (3% vs 0.3%; $p<0.0001$). However, these two groups were not equivalent since the EIA group had a greater proportion of individuals with SS defined by ACR criteria (49% vs 44%; $p = 0.0113$). Anti-SSB was more commonly present in low titre (levels of 1 to ≤6 index units by EIA, and 1 to ≤8 AI units by MFIA) when occurring alone than when associated with anti-SSA of any titre (91% vs 49%; $p<0.0001$).

The prevalence of key SS phenotypic features differed significantly among the three anti-SSA/anti-SSB serological groups (table 2). These differences stemmed primarily from comparisons of the group with anti-SSA with or without anti-SSB and the groups of patients with anti-SSB alone or with negative SSA/SSB. In contrast, the frequency of these disease markers was statistically comparable between the groups with only anti-SSB and those with negative SSA/SSB. The only exceptions were low unstimulated whole saliva flow (UWSF) rate, dry mouth symptoms and antinuclear antibody positivity that were actually more prevalent among the participants with negative SSA/SSB than in those with anti-SSB alone.

Key phenotypic features of SS expressed as continuous variables also differed by SSA/SSB serological profile (table 2). The median ocular staining score (OSS) and focus score (FS) were higher and Schirmer test and UWSF values were lower among participants with anti-SSA with or without anti-SSB than among participants with only anti-SSB or with negative anti-SSA/anti-SSB. The differences between the two groups with respect to these SS phenotypic features were all statistically significant ($p<0.0001$). Levels of OSS and FS did not differ significantly between the group with only anti-SSB and that with negative anti-SSA/anti-SSB. In contrast, the group with only anti-SSB had significantly higher median Schirmer test and UWSF results than the group lacking both antibodies, opposite to what was expected.

With a logistic regression analysis of the joint effects of anti-SSA and anti-SSB on selected SS phenotypic features, we quantified the between-group differences observed in table 2 and showed that these are not explained by confounding by age, race/ethnicity or gender. Results are summarised in table 3 as adjusted ORs comparing the odds of occurrence for each feature between specified groups defined using anti-SSA and anti-SSB status.

Positive SSA/SSB serology was present in 1138 (76%) of those participants who met the ACR criteria and in 1067 (73%) of those who met the AECG criteria for SS. In each of these two groups, 52 participants had anti-SSB alone (ACR: 3%; AECG: 4%).

Classification of subjects with SS was dependent on the presence of anti-SSB alone (due to the absence of the positive lip biopsy criterion) in 33 (2.2%) of the subjects using the ACR criteria and in 34 (2.3%) using the AECG criteria.

DISCUSSION

In this large registry of individuals with suspected or established SS, anti-SSB in the absence of anti-SSA was found in 2% and had no association with SS phenotypic features compared

with those who lacked both antibodies. This is an important and novel observation since current SS classification schema include these antibodies as criteria but do not differentiate among the various possible anti-SSA and anti-SSB serological profiles, giving equal weight to all.[1213]

The rare detection of the 'anti-SSB alone' pattern of reactivity among the SICCA registrants may be a function of the assay methodology or a true entity The Bio-Rad Bioplex 2200 MFIA has greater sensitivity for anti-SSB relative to double immunodiffusion and HEp-2 immunofluorescence assays.[1116] In our cohort, anti-SSB alone was 10 times more commonly detected with this MFIA than with EIA. A false-positive reaction to SSB may result from low-titre, weak-affinity antibodies with no pathological relevance[15] or cross-reacting antibodies, such as those to histones, cardiac myosin and spectrin.[17–19] Recently, Danda *et al*[20]analysed 29 anti-Ro60 (SSA)-negative/anti-La (SSB)-positive sera from their cohort of 468 patients with SS; in 25, the sera were unequivocally negative for anti-Ro60 with four different assay methods, including an immunofluorescent assay using HEp-2000 cells transfected with human Ro60.

As part of a separate study, 5 of the 74 SSB-positive/SSA-negative sera from the SICCA cohort were assayed in the Johns Hopkins Rheumatic Diseases Research Core Center for antibodies to Ro52 and SSB using ELISA (QUANTA Lite, Inova Diagnostics) and for R06O by immunoprecipitation using in vitro transcription/translated protein.[2122] Each of the sera was negative for anti-Ro52 and anti-Ro60 antibodies. Anti-SSB antibodies were confirmed by ELISA in three of the sera, with weak positive (20–39 units) results in two and moderate positive (40–80 units) in one. These findings support those of Danda *et al* and suggest that the SSB-positive/SSA-negative serological profile can be a true positive, although the anti-SSB reactivity is typically low titre.

The presence of anti-SSA and/or anti-SSB is a mandatory criterion in the absence of a 'positive' labial salivary gland (LSG) biopsy in the 2002 AECG and 2012 ACR criteria sets.[1213] The findings in the current study have implications for the serological diagnosis of SS as stipulated in these criteria. Thus, our data argue that the SSB-positive/SSA-negative antibody profile should be interpreted cautiously in a patient with suspected SS. In this circumstance, additional support for the diagnosis is advisable in the form of a LSG biopsy showing focal lymphocytic sialadenitis with an FS ≥1. Elimination of anti-SSB alone as a serological criterion for SS should be considered by the international community as it prepares new consensus criteria for SS; the impact would be limited to only 2% of subjects currently classified with SS by the AECG and ACR criteria sets.

Strengths of our study include the large size and geographic heterogeneity of the SICCA cohort. Inclusion criteria for the registry were intentionally broad to ensure representation of the spectrum of individuals evaluated for SS. Each participant underwent a uniform evaluation with all LSG biopsies being read centrally and SSA/SSB serological testing being performed in the same clinical laboratory, thereby assuring results representative of those encountered in clinical practice.

A limitation of the study is the small number of individuals with anti-SSB alone, resulting in lower power for statistical analyses related to this group. Nevertheless, this is the largest number studied to date. Our analysis was also cross sectional.

In conclusion, the presence of anti-SSB alone is rare and has no association with key SS phenotypic features. These findings are particularly relevant with the advent of MFIAs for anti-SSA/anti-SSB testing since they may reveal low levels of anti-SSB with no clinical significance in the evaluation of a patient with suspected SS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Sociodemographic characteristics of 3297 SICCA participants *

Age, years; median (range)	54 (21–90)
Women, %	3001 (91)
Ethnicity, no.	
Caucasian	1825 (55)
Asian	866 (26)
Hispanic	307 (9)
African	81 (2)
Native American	30 (1)
Multirace	113 (2)
Unspecified	75 (2)
Recruitment site	
USA	1233 (37)
Denmark	588 (18)
Argentina	431(13)
Japan	351(11)
China	297 (9)
UK	292 (9)
India	105 (3)
Smoking	
Current	319 (10)
Former	1053 (32)
Never	1918 (58)

* Values are the number (%), unless otherwise stated; data are not available regarding smoking status for seven participants.

SICCA, Sjögren's International Collaborative Clinical Alliance.

Table 2

Phenotypic characteristics (categorical variables) of 3297 SICCA participants by anti-SSA and anti-SSB reactivity patterns

	Antibody reactivity pattern			p Values*		
	Group A: anti-SSA with/ without anti-SSB (N=1162)	Group B: anti-SSB alone (N=74)	Group C: neither anti-SSA nor anti-SSB (N=2061)	Overall	Group A vs group B	Group B vs group C
Categorical variables	n (%)†	n (%)†	n (%)†			
Maximum OSS ≥3	1030 (89)	44 (59)	1365 (66)	<0.0001	<0.0001	0.2607
Schirmer ≤5 mm/5 min (mean value for both eyes)	622 (55)	11 (15)	480 (23)	<0.0001	<0.0001	0.0933
UWSF <0.1 mL/min	711 (61)	25 (34)	968 (47)	<0.0001	<0.0001	0.0321
WBC $\leq 4.0\times10^9$/L	302 (26)	4 (5)	134 (7)	<0.0001	<0.0001	1.0
ANA	911 (78)	17 (23)	744 (36)	<0.0001	<0.0001	0.0205
ANA ≤1:320	719 (62)	5 (7)	302 (15)	<0.0001	<0.0001	0.0625
IgG >14.45 g/L	738 (64)	5 (7)	265 (13)	<0.0001	<0.0001	0.1529
Positive RF	761 (65)	9 (12)	361(18)	<0.0001	<0.0001	0.2752
C4 <0.16 g/L	234 (20)	5 (7)	196 (10)	<0.0001	0.0035	0.5450
FL or FL/S sialadenitis with FS ≥1‡	829 (71)	19 (26)	427 (21)	<0.0001	<0.0001	0.3088
Dry eye symptoms	961 (83)	61 (82)	1802 (88)	0.0005	0.8751	0.2072
Dry mouth symptoms	1037 (89)	61 (82)	1864 (91)	0.0421	0.0816	0.0255
Continuous variables§						
FS	2.6 (0.1–12.5)	1.0 (0.2–10.2)	0.9 (0.1–13.5)	<0.0001	<0.0001	0.6295
OSS (maximum for both eyes)	8 (0–12)	3 (0–12)	4 (0–12)	<0.0001	<0.0001	0.1561
Schirmer (mean for both eyes)	6 (0–35)	14 (0–32)	10 (0–35)	<0.0001	<0.0001	0.0040
UWSF, mL/5 min	0.087 (0–12.7)	0.391 (0–4.0)	0.215 (0–12.6)	<0.0001	<0.0001	0.0010

*Fisher's exact test, no correction for multiple comparisons.

†Column percent. Denominator may vary slightly in case of missing observations for some phenotypic features.

‡'Positive' lip biopsy.

§Values are the median (range).

ANA, antinuclear antibody; C4, complement 4 protein; FL/S, focal lymphocytic/sialadenitis; FS, focus score; IgG, immunoglobulin G; OSS, ocular surface staining score; RF, rheumatoid factor; SICCA, Sjögren's International Collaborative Clinical Alliance; SSA, Sjögren's syndrome A; SSB, Sjögren's syndrome B; UWSF, unstimulated whole saliva flow rate.

Table 3

Effect of anti-SSA and/or anti-SSB status on each phenotypic feature of SS [*]

Phenotypic feature of SS (dependent variables for models 1–6)	Anti-SSA and anti-SSB vs anti-SSA alone	p Value	Anti-SSA alone vs absence of both anti-SSA and anti-SSB	p Value	Anti-SSB alone vs absence of both anti-SSA and anti-SSB	p Value
1. FS ≥1 vs FS <1 or no FLS	4.5 (3.4 to 5.9)	<0.0001	4.3 (3.5 to 5.3)	<0.0001	1.4 (0.8 to 2.4)	0.2
2. OSS≥3 vs <3	4.2 (2.8 to 6.3)	<0.0001	2.0 (1.5 to 2.5)	<0.0001	0.9 (0.5 to 1.4)	0.5
3. Schirmer ≤5 mm/5 min vs<5 mm/5min	1.8 (1.4 to 2.3)	<0.0001	1.7 (1.3 to 2.1)	<0.0001	0.7 (0.4 to 1.3)	0.3
4. UWSF<0.1 mL/min vs ≥0.1 mL/min	2.2 (1.7 to 2.8)	<0.0001	1.5 (1.2 to 1.8)	<0.0001	0.7 (0.4 to 1.1)	0.1
5. RF (+ vs −)	6.1 (4.7 to 7.9)	<0.0001	3.2 (2.6 to 4.0)	<0.0001	0.7 (0.4 to 1.5)	0.4
6. IgG>14.45 g/L vs ≤14.45 g/L	5.6 (4.2 to 7.3)	<0.0001	4.1 (3.2 to 5.2)	<0.0001	0.7 (0.3 to 1.7)	0.4

[*] Values are ORs and 95% CIs estimated using logistic regression models (1–6) that were adjusted for age, gender and ethnicity.

FLS, focal lymphocytic sialadenitis; FS, focus score; IgG, immunoglobulin G; OSS, ocular surface staining score; RF, rheumatoid factor; SSA, Sjögren's syndrome A; SSB, Sjögren's syndrome B; UWSF, unstimulated whole saliva flow rate.